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DATA EVALUATION RECORD

STUDY TYPE: Rodent and Human *In Vitro* Dermal Penetration Study – Rat and Human Non-Guideline

PC CODE: 025002 **DP BARCODE:** 339369

TEST MATERIAL (PURITY): Creosote (98.5%)

SYNONYMS: AWPA P1-P13 Creosote; American Wood Preserves Association P1-P13 Creosote

CITATION: Fasano, W. (2007) AWPA P1-P13 Creosote: In vitro kinetics in rat and human skin. E.I. du Pont de Nemours and Company, Haskell[SM] Laboratory for Health and Environmental Sciences, Newark, Delaware. Laboratory Project ID: DuPont-21647, April 30, 2007. MRID 47179502.Unpublished.

SPONSOR: The Creosote Council III, P.O. Box 160, Valencia, Pennsylvania 16059.

EXECUTIVE SUMMARY: In an *in vitro* dermal absorption study using rat and human skin preparations (MRID 47179502), AWPA P1-P13 Creosote (98.5% a.i.) spiked with eight radiolabeled target chemicals was applied to 6 rat and 6 human skin preparations mounted in individual static diffusion cells at a nominal dose of 10,700 µg creosote/cm^2 (1 µCi/skin). Samples were collected from the receptor chambers at 0.5, 1, 2, 4, and 8 hours post-application. The skin was washed 8 hours post-application and the study terminated. The amount of the applied dose absorbed was considered to be the amount of radioactivity measured in the receptor fluid.

The mean total recovery of the applied dose for the rat and human skin was 78.3% and 83.9%, respectively. The mean absorbed dose was 15.1% for the rat skin and 3.34% for the human skin. The mean rate of penetration was 85.3 µg equiv/cm^2/hr for the rat skin and 19.7µg equiv/cm^2/hr for the human skin. The mean rate of penetration and the mean total penetration for the rat skin were approximately 4.3 times and 4.4 times greater than that for the human skin, respectively.

This *in vitro* study using rat and human skin sample preparations is currently not acceptable for regulatory purposes. Additional data must be provided in order to consider the data in this study for assessment of dermal absorption in conjunction with the submitted in vivo dermal absorption study (MRID 47179501). Deficiencies are listed at the end of this review.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were included. The GLP statement notes the following exception to compliance with 40 CFR Part 160: the chemical and radiochemical concentration and the radiochemical purity of the selected chemicals, which were spiked into the creosote test substance, was based on the certificates of analyses provided by the sponsor and vendors and the verified radioactivity per volume.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:	AWPA P1-P13 Creosote
Description:	Creosote: dark, amber-colored liquid Radiolabeled chemicals: benzo(a)pyrene (toluene solution); 2-methylnaphthalene (solid); fluoranthene (methanol solution); anthracene (toluene solution); naphthalene-benzene (solid); phenanthrene (solid); biphenyl (toluene solution); pyrene (solid)
Lot/batch #:	Creosote test substance: not provided Radiolabeled chemicals: benzo(a)pyrene (033H9241); 2-methylnaphthalene (050K9424/25); fluoranthene (054K9630); anthracene (018H9432/33); naphthalene-benzene (068H9600/01); phenanthrene (111K9412/13); biphenyl (115F9247); pyrene (079H9662/63)
Purity:	Creosote: 98.5% a.i. Radiolabeled chemicals: 2-methylnapthalene (99.707%); others (not provided)
Compound stability:	Minimum of 4 years (storage conditions not specified)
CAS # for TGAI:	8001-58-9
Structure:	Not applicable; a mixture of aromatic hydrocarbons
Vehicle/Solvent used:	Not applicable; test substance applied as neat creosote
Radiolabeling:	benzo(a)pyrene-7-^{14}C; 2-methylnaphthalene- 8-^{14}C; fluoranthene-3-^{14}C; anthracene-1,2,3,4,4A,9A-^{14}C; naphthalene-benzene-UL-^{14}C; phenanthrene-9-^{14}C; biphenyl-UL-^{14}C; pyrene-4,5,9,10-^{14}C
Specific Activity:	benzo(a)pyrene (26.6 mCi/mmol); 2-methylnaphthalene (8.5 mCi/mmol); fluoranthene (45 mCi/mmol); anthracene (20.6 mCi/mmol); naphthalene-benzene (31.3 mCi/mmol); phenanthrene (8.2 mCi/mmol); biphenyl (7.6 mCi/mmol); pyrene (55 mCi/mmol)
Radiochemical Purity:	benzo(a)pyrene (94.781%); 2-methylnaphthalene (not provided);

	fluoranthene (99.523%); anthracene (97.524%); naphthalene-benzene (99.119%); phenanthrene (99.623%); biphenyl (100%); pyrene (97.186%)
Source:	Creosote: sponsor Selected radiolabeled chemicals: Sigma-Aldrich Co., St. Louis MO

2. **Relevance of test material to proposed formulation(s):** The creosote test material was applied neat, spiked with eight selected radiolabeled chemicals.

3. **Test animals / rodent skin:**

Species:	Rat, male
Strain:	Sprague-Dawley Crl:CD(SD)
Age/weight at study initiation:	6-8 weeks; weight not specified
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Not specified
Diet:	Not specified
Water:	Not specified
Environmental conditions:	Not specified
Acclimation period:	Quarantined prior to use; length of quarantine period not specified
Comments:	Rats were sacrificed by CO_2 asphyxiation and the dorsal region carefully shaved using clippers. Any animals showing obvious abrasion were not used. The shaved area was excised, placed on an aluminum pan (marked with the corresponding rat ID number), held briefly on wet ice, then frozen at approximately -20°C until prepared for use.

4. **Human skin:** Samples of human cadaver skin were obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA). The source and identity of the skin sample (sex, anatomical locale, approximate age of donor) were reported to have been documented in the study records, but were not included in the report.

B. STUDY DESIGN:

1. **Dose:**

Rationale: Not provided.

Nominal doses: The nominal doses are summarized in Table 1.

Table 1: Nominal Doses of Creosote[a]

Species	Group	Creosote Concentration (µg total creosote/mL)[b]	Volume of Creosote Applied to Skin (µL)	Skin Dose Level (µg creosote/cm^2)[c]	Number of Skin Preparations	µCi/skin
Rat	A	1,070,000	6.4	10,700	6	1.0
Human	B	1,070,000	6.4	10,700	6	1.0

[a] Data were obtained from page 10 of the study report.
[b] Based on a density of 1.07 gram total creosote/mL
[c] Exposure area of 0.64 cm^2

Actual doses: 1.05 µCi/dose (Groups A and B)

Dose volume: 10 µL/cm^2 skin

Duration of exposure (time from dose to skin wash): 8 hours

Number of skin preparations/group: 6 (representing at least 3 individuals/group)

2. **Skin membrane preparation:** The frozen rat and human skin samples were thawed at room temperature. Full thickness skin was dermatomed to approximately 450 µm. Each skin sample was placed on a labeled aluminum pan and refrigerated at 1-10°C until ready for use.

3. **Diffusion cell preparation:** Glass (static) diffusion cells were used. Each skin preparation was mounted, stratum corneum uppermost, onto the top of the receptor chamber, which was filled with saline receptor fluid. The donor chamber was placed over the skin and clamped in place. The exposed skin surface area was 0.64 cm^2. The receptor fluid was stirred throughout the exposure period using a magnetic stir bar.

4. **Membrane integrity assessment, membrane equilibration, and pre-treatment procedures:** Prior to application of the test substance, each membrane was assessed for integrity by measuring electrical impedance. Membranes were removed from refrigeration and hydrated in 0.9% saline for approximately 15 minutes. Each membrane was then mounted onto the top of a receptor chamber, which was filled with 0.9% saline. The donor chamber was clamped in place, filled with 0.9% saline, and the membrane allowed to equilibrate for approximately 30 minutes at approximately 32°C using a re-circulating water bath system. An impedance measurement was then taken. Membranes with an impedance of ≥6 kΩ (rat) and ≥17 kΩ (human) were considered intact and retained for use in the study. The procedure was followed until a minimum of 6 skin preparations represented by at least 3 individuals per species were obtained. Saline in each donor chamber was removed and discarded; saline in the receptor chamber was reduced to approximately one-half of the total volume.

   Acceptable membranes were kept at approximately 32°C overnight, without occlusion of the donor chamber, prior to dose application; the receptor chamber sampling arm remained

occluded with Parafilm®. Following overnight equilibrium, the contents of the receptor chamber were discarded and refilled with 50%(v/v) ethanol in deionized water. Prior to application of the test substance, a 50µL sample was taken from the receptor fluid (pre-treatment background) and replaced with an equal volume of fresh receptor fluid.

5. **Dose application and determination:** The test substance was applied to the exposed skin surface in the donor chamber as a single application distributed evenly over the exposure area. The donor chamber opening was then occluded with Parafilm® for the duration of the exposure period.

   The homogeneity of the radioactivity in the test substance and the amount of radioactivity administered were determined by counting replicate aliquots (mock doses) and using the mean value as the amount of radioactivity applied. The total amount of creosote applied to the skin (total µg equivalents) was based on the amount of radioactivity applied (mock dose) and the calculated specific activity (0.37µCi/mg).

6. **Sample collection:** Duplicate 50 µL samples were collected from the receptor chambers at 0.5, 1, 2, 4, and 8 hours post-application. Receptor fluid was placed directly into liquid scintillation vials. The volume of the removed receptor fluid was replaced with fresh receptor fluid. Other than at sampling, the receptor chamber arm remained occluded with Parafilm®.

   At 8 hours post-application, the surface of the skin was washed three times 1 with a 2% Ivory® soap solution, followed by a rinse with 1 mL of deionized water. The washes and rinse were collected into a liquid scintillation vial. The donor chamber remained clamped in place during the procedure.

   Following the wash and rinse, the donor chamber was removed and rinsed with approximately 5 mL of acetonitrile directly into a liquid scintillation vial. The skin membrane was removed from the receptor chamber and tape-stripped five times to remove the stratum corneum using Leukotape® P. Each piece of tape was placed into an individual glass vial and extracted with acetonitrile. The remaining piece of skin was placed in a glass vial for digestion.

7. **Sample preparation and analysis:** The receptor fluid samples, donor chamber rinse, skin wash, and tape strips were not processed further. Ultima Gold™ XR scintillation cocktail was added to each vial and the samples analyzed for radioactivity.

   The skin was digested in Soluene®-350 at approximately 60°C with constant shaking. Hionic-Fluor™ liquid scintillation cocktail was added to each vial and the samples analyzed for radioactivity.

   Samples were counted in a Packard liquid scintillation counter for 10 minutes or until 160,000 disintegrations were accumulated. The limit of detection of each sample was taken as twice the background disintegration rate obtained from blank samples.

   Statistical evaluation ($p \leq 0.05$) of the group data was performed using Grubb's outlier test. An absorption profile was produced by plotting the cumulative amount of ^{14}C-creosote

equivalents in the receptor chamber at each collection timepoint, (adjusted for total receptor chamber volume) against time (hours).

II. RESULTS:

A. TOTAL ABSORBED DOSE: The total absorbed dose was calculated as the percent of the applied dose found in the receptor fluid. The mean percent recovery of radioactivity for each analyzed medium, and the mean absorbed, absorbable, and unabsorbed doses are summarized for the rat and human skin in Table 2.

Table 2: Percent Recovery of the Applied Radioactive Dose for Each Medium[a]

Medium	Percent of Applied Dose (mean±SD)	
	Rat Skin (Group A) (n=6)	Human Skin (Group B) (n=6)
Receptor fluid	15.1±3.64	3.38±1.03
Total absorbed dose	**15.1±3.64**	**3.38±1.03**
Receptor fluid	15.1±3.64	3.38±1.03
Tape-stripped skin	19.2±6.82	0.86±0.26
Total absorbable dose	**34.3±6.84**	**4.24±1.07**
Skin wash	12.8±2.33	70.3±7.52
Donor chamber	7.52±2.44	1.89±0.75
Tape strips	23.6±4.60	5.33±0.98
Total unabsorbed dose	**44.0±5.98**	**79.7±4.08**
Total Recovery	**78.3±2.44**	**83.9±3.68**

[a] Data were obtained from page 19 (Table 2) of the study report.

The recovery of the applied dose was slightly low, but similar, for the rat and human skin (78.3±2.44% and 83.9±3.68%, respectively). For the rat skin, the highest percentage of the applied dose was found in the tape strips (23.6±4.60%), followed by the tape-stripped skin (19.2±6.82%). For the human skin, the majority of the applied dose was found in the skin wash (70.3±7.52%). The total absorbed dose for the rat and human skin was 15.1±3.64% and 3.38±1.03%, respectively.

B. PENETRATION KINETICS: The penetration kinetics for the test substance are summarized in Table 3.

Table 3: Penetration Kinetics of ^{14}C-Creosote[a]

Time	cumulative µg equiv/cm^2 (mean±SD)	
	Rat Skin (Group A) (n=6)	Human Skin (Group B) (n=6)
0.5	24.0±14.1	5.11±1.85
1	74.0±31.9	8.38±4.56
2	189.9±63.4	27.1±12.5
4	373.6±103.4	66.9±24.6
8	665.8±161.0	149.7±45.7
Penetration Rate, 0.5-8 hr[b] (µg equiv/cm^2/hr)	**85.3**	**19.7**

[a] Data were obtained from page 18 (Table 1) of the study report.

[b] Slope of the mean data, 0.5-8 hr

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** During the 8-hr exposure period, the radioactivity penetrated through the rat skin approximately 4.3 times faster (85.3 μg equiv/cm^2/hr) than through the human skin (19.7 μg equiv/cm^2/hr). The total penetration of radioactivity at the end of the exposure period was 4.4 times greater for the rat skin (665.8 μg equiv/cm^2) than for the human skin (149.7 μg equiv/cm^2).

Washing of the skin at the end of the exposure removed 12.8% and 70.3% of the applied creosote test substance from the rat and human skin, respectively, which reflected the greater rate and extend of total penetration for rat skin. A significant portion of the unabsorbed dose for the rat skin (44%) was contained in the stratum corneum (23.6%); a minor portion of the unabsorbed dose for human skin (79.7%) was found in the stratum corneum (5.33%).

The total absorbable dose was 34.3% and 4.24% for the rat and human skin, respectively. The results confirm that creosote penetrated through rat skin at a faster rate and to a greater extent than for human skin, and that the total absorbable dose was 8 time greater for rat skin than for human skin.

B. **REVIEWER COMMENTS:** Based on the study results, the investigator's conclusions are correct, with one qualification. The study is not typical in that it determined the dermal absorption of radiolabeled chemicals that were added to the test substance, rather than radiolabeled components of the test substance itself. Any conclusions that are reached regarding absorption apply to the absorption of the radiolabeled chemicals added to the creosote, and not to the absorption of creosote itself.

Although the percent recovery of the applied dose was similar for both the rat and human skin preparations, it was slightly low. The investigators suggest that chemical instability and subsequent volatilization from the wash, the skin during tape-stripping, and/or from the tape strip sections prior to solvent extraction may have occurred. This has been previously observed in their laboratory for water-insoluble (radiolabeled) chemicals.

C. **STUDY DEFICIENCIES:** This is a non-guideline study. The following deficiencies, based on the guidelines for *in vivo* dermal absorption studies (OPPTS 870.7600), are potentially applicable.

Major study deficiencies were:

- Only one exposure duration was evaluated.
- Only one dose level was evaluated.
- Data on solubility as a rate-limiting factor for in vitro absorption was not provided.
- A 24 hour time point measurement was not conducted.

Minor deficiencies were:

- The lot/batch number of the creosote test substance was not provided.
- The time between test substance preparation and application was not provided.
- Storage conditions for the test substance were not provided.
- A study protocol was not included.

In addition, the following deficiency specific to the *in vitro* study was noted:

- The source and identity of the human skin samples (sex, anatomical location, age) were not included in the study report, although they were reported to have been documented in the study records.

D. STUDY CLASSIFICATION: This *in vitro* study using rat and human skin sample preparations is currently not acceptable for regulatory purposes. Additional data must be provided in order to consider the data in this study for assessment of dermal absorption in conjunction with the submitted in vivo dermal absorption study (MRID 47179501).